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Original Contribution

Association Between Left Ventricular Mass and Telomere Length in a Population Study

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Experimental studies have implicated telomere dynamics in cardiomyocyte size and replication potential; shorter telomeres mark attenuated proliferation and increased apoptosis. The authors examined whether this translates into an impact of telomere length (TL) on left ventricular (LV) mass in the general population. In 334 randomly selected Flemish participants (mean age = 46.5 years; 52.5% women), they measured TL in circulating leukocytes using quantitative polymerase chain reaction, expressing it as telomere/genomic DNA ratio (T/S). After a median 7.4 years of follow-up (interquartile range, 6.2–8.5) during 1996–2007, they measured LV mass by echocardiography. In multivariable-adjusted analyses accounting for sex, age, body weight and height, systolic blood pressure, and antihypertensive drug use, LV mass and LV mass index significantly increased with mean leukocyte TL in the entire population and in the 198 normotensive subjects. For a 1-standard-deviation increment in T/S ratio, LV mass (mean = 170 g) and LV mass index (mean = 92 g/m^2) increased by 5.20 g ($P = 0.003$) and 2.70 g/m^2 ($P = 0.004$), respectively, in all subjects and by 8.03 g ($P = 0.0001$) and 3.74 g/m^2 ($P = 0.0007$) in normotensive subjects. There were corresponding associations with LV wall thicknesses ($P < 0.007$) but not LV internal diameter ($P = 0.26$) in normotensive subjects. Longer mean leukocyte TL is associated with increased LV mass, particularly in normotensive subjects. This association could have a biologic basis related to the role of TL in determining cardiomyocyte size and replication potential.

heart ventricles; myocytes, cardiac; population; telomere

Abbreviations: CI, confidence interval; LV, left ventricular; SD, standard deviation; TERT, telomerase reverse transcriptase.

Telomeres—the specialized DNA-protein structures capping the ends of eukaryotic chromosomes—are essential for maintaining chromosomal stability and integrity (1). Telomeres consist of a large number of tandem repeats of a simple DNA sequence (TTAGGG in humans), and telomere length is an important determinant of telomere function (2). Telomere length depends on many factors, including inheritance, cellular replicative history, and the activity level of telomerase—a reverse transcriptase consisting of an RNA component and a catalytic protein component, telomerase reverse transcriptase (TERT), that has the ability to add telomere repeats (2, 3). In the absence of telomerase, because DNA polymerase cannot fully complete the replication of the 3′ end of linear DNA, telomeres progressively shorten with repeated cell divisions (4). In many types of cells, senescence and subsequent cell death often occurs when the mean telomere length reaches a critical value (5, 6).

Previously, the number of muscle cells in the mammalian myocardium was thought to be defined at birth. However, recent evidence from both experimental and human studies suggests that the myocardium consists of a heterogeneous population of cardiomyocytes, characterized by ongoing cell death and cell regeneration during the entire life span (7–9). Furthermore, several observations suggest an important role for telomeres and telomerase in this process. With aging, telomerase knockout mice showed shortening of telomeres, attenuated proliferation and increased apoptosis of

cardiomyocytes, and a higher risk of cardiac remodeling and left ventricular (LV) failure (10, 11). On the other hand, enhanced expression of TERT in rat cardiomyocytes preserved telomerase activity and telomere length and induced cardiomyocyte hypertrophy without fibrosis or loss of function (12). These observations suggest that telomere length may be an important determinant of the rate at which cardiomyocyte attrition occurs with age. In turn, because cardiomyocytes are the major contributor to LV mass, this could affect LV mass. Therefore, we tested the hypothesis that telomere length predicts LV mass in a random sample of the general population.

MATERIALS AND METHODS

Study participants

Study participants were from the Flemish Study on Environment, Genes and Health Outcomes, consisting of a random population sample, stratified by sex and age, selected from a geographically defined area in northern Belgium. Officials from 7 municipalities gave us listings of all inhabitants, sorted by address. Households, defined as all subjects living at the same address, were the sampling unit. We numbered households consecutively and generated a random-number list through the use of a SAS random function (SAS Institute Inc., Cary, North Carolina). Households with a number matching the list were invited to participate. Further details on the sampling frame are given elsewhere (13).

Initial assessment of the study population took place between 1996 and 2000 and involved 638 subjects. Follow-up took place between 2005 and 2007. The participation rate among the subjects contacted in 2005–2007 averaged 72.3%. Blood for the follow-up DNA extraction could not be obtained from 260 participants, because they did not consent ($n = 145$), had died ($n = 24$), were bedridden or institutionalized ($n = 19$), or had moved out of the area or could not be reached ($n = 72$). Thus, we reexamined 378 former participants at our field center, including echocardiography. We excluded 32 subjects from the present analysis because of LV remodeling due to myocardial infarction or coronary revascularization ($n = 9$), valvular heart disease ($n = 16$), atrial fibrillation ($n = 5$), or an artificial pacemaker ($n = 2$). We discarded a further 12 subjects because the echocardiogram ($n = 5$) or DNA for telomere measurements ($n = 7$) was of insufficient quality. Thus, the number of participants statistically analyzed totaled 334.

The Ethics Committee of the University of Leuven (Leuven, Belgium) approved the study protocol (baseline and follow-up), and participants provided written informed consent.

Clinical measurements

We used a validated questionnaire to inquire about lifestyle, medical history, medication use, smoking, and alcohol drinking. The questionnaire also obtained detailed information on the total number of hours spent in sports and occupational activities, including school attendance for younger people. With the use of published tables, we estimated the amount of energy spent in physical activity from body weight, time devoted to work and sports, and types of physical activity reported (14). Trained nurses measured anthropometric characteristics and took blood pressure 5 times consecutively to the nearest 2 mm Hg after the subject had been seated for at least 5 minutes. Normotension was defined as untreated blood pressure below 140 mm Hg (systolic) and below 90 mm Hg (diastolic) at both baseline (1996–2000) and follow-up (2005–2007). Hypertension was defined as a blood pressure equal to or higher than these thresholds or the use of antihypertensive medication at baseline, follow-up, or both (15). Body mass index was calculated as weight in kilograms divided by the square of height in meters.

Echocardiography at follow-up assessment (2005–2007)

All echocardiograms were performed and analyzed by a single experienced physician (T. K.) using a Vivid 7 Pro device (GE Vingmed Ultrasound, Horten, Norway) interfaced with a 2.5- to 3.5-MHz phased-array probe as described elsewhere (16). Briefly, with the subject in partial left decubitus and breathing normally, parasternal long and short axes and apical 4- and 2-chamber long-axis views were obtained, together with a simultaneous electrocardiographic signal. All recordings included at least 5 cardiac cycles, and results were digitally stored for off-line analysis. M-mode echocardiograms of the left ventricle were recorded from the parasternal long-axis view under control of the 2-dimensional image. The ultrasound beam was positioned just below the mitral valve at the level of the posterior chordae tendineae.

Images were analyzed at a work station on a computer using EchoPac software, version 4.0.4 (GE Vingmed Ultrasound). To improve accuracy, results from 3 heart cycles were averaged. The LV internal diameter and interventricular septal and posterior wall thickness were measured at end-diastole from 2-dimensional guided M-mode tracings, according to current guidelines (17). End-diastolic LV dimensions were used to calculate LV mass by means of an anatomically validated formula (18). The ejection fraction was calculated as (end-diastolic volume − end-systolic volume)/LV end-diastolic volume. LV mass index was defined as LV mass divided by body surface area, calculated as body weight$^{0.425}$ (in kg) × body height$^{0.725}$ (in cm) × 0.007184. Relative wall thickness was calculated as (2 × posterior wall)/LV internal diameter at end-diastole. LV concentric remodeling was defined as a relative wall thickness exceeding 0.43. LV hypertrophy was defined as an LV mass index exceeding 110 g/m^2 in women and 125 g/m^2 in men (19). To determine intraobserver reproducibility, the echocardiographer (T. K.) analyzed the echocardiograms of 17 subjects twice. The intraobserver reproducibility coefficient of a measurement was the 2-standard-deviation (SD) interval around the mean of the relative differences across pairwise readings. The intraobserver reproducibility was 2.2% for LV internal end-diastolic diameter, 4.6% for LV wall thickness, and 4.3% for LV mass.

Measurement of telomere length in DNA obtained at baseline (1996–2000) and at follow-up (2005–2007)

Genomic DNA was extracted from peripheral blood, using the phenol method. To measure telomere length, we used real-time quantitative polymerase chain reaction as described previously (20, 21). We determined the relative ratio of telomere repeat copy number (T) to single-copy gene copy number (*36B4* gene; S), with all samples being compared with the same reference DNA sample. Therefore, telomere length was expressed as the T/S ratio. Samples were analyzed in duplicate wells together with a duplicate calibrator well (K562 cell line genomic DNA) and a no-template control well (to test for contamination). Determination of T and S quantities was performed by means of RotorGene comparative quantitation software (Corbett Research, Sydney, Australia) (21). The coefficients of variation between duplicate measurements within the same run were 1.9% (T), 1.4% (S), and 2.6% (T/S). To test for interrun variation, we reanalyzed 12.5% of the samples on a separate day; the coefficient of variation was 4.2%.

Statistical analysis

For database management and statistical analysis, we used SAS software, version 9.1. We investigated associations between variables by means of simple and multiple linear regression. For continuous LV traits, we adjusted the models for possible covariables based on our previous publications (22) with known physiologic relevance for left ventricle structure and confirmed in the present study by stepwise linear regression. We searched for variables associated with T/S ratio using stepwise linear regression. The significance level for variables to enter and stay in the regression models was set at $P < 0.05$. Common covariables significantly associated with the explanatory variable (T/S ratio) and the outcome variables (LV traits) were age and sex. We tested the association of continuous traits with the T/S ratio by use of a mixed model. This technique allows accounting for covariables as well as for the nonindependence of observations within families. We expressed multivariable-adjusted effect sizes for a 1-SD increase in the explanatory variables. To calculate residuals, we adjusted the T/S ratio for age and sex and LV traits for age, sex, anthropometric characteristics, and systolic blood pressure. All P values were for 2-sided tests.

RESULTS

Characteristics of participants

The cohort with telomere measurements at baseline included 334 subjects, of whom 181 (54.2%) were women and 136 (40.7%) were persons with hypertension (treated but not controlled, 38 patients; treated and controlled, 36 patients; untreated, 62 patients). The median interval between the baseline T/S measurement and cardiac phenotyping was 7.4 years (interquartile range, 6.2–8.5). Table 1 shows the clinical characteristics of participants in the entire study population and those subjects who were normotensive at both baseline (1996–2000) and follow-up (2005–2007) ($n = 198$) or hypertensive at baseline, follow-up, or both ($n = 136$). Table 1 also shows corresponding echocardiographic characteristics. Overall, 28 subjects (8.4%) had LV hypertrophy (Table 1). In the normotensive group, 4 subjects (2.0%) had eccentric LV hypertrophy and 12 subjects (6.1%) had a normal LV mass but increased relative wall thickness (concentric remodeling) (Table 1). In hypertensive patients, concentric and eccentric hypertrophy and concentric remodeling were present in 4.4%, 13.2%, and 17.6%, respectively (Table 1).

Both at baseline and at follow-up, age was a significant determinant of the T/S ratio ($P < 0.0001$; Figure 1), accounting for 14.2% and 16.0% of the variance, respectively. In cross-sectional analyses of the baseline and follow-up data, estimates of the annual decrease in the T/S ratio were −0.0067 (95% confidence interval (CI): −0.0084, −0.0050) and −0.0070 (95% CI: −0.0086, −0.0054), respectively. The longitudinally measured annual T/S attrition rate based on the differences between baseline and follow-up averaged −0.0077 (SD, 0.018) per year (95% CI: −0.0086, −0.0054), showing concordance with the cross-sectional estimates. The 5th–95th percentiles of the T/S ratio changes ranged from −0.033 to 0.023, reflecting that in some subjects telomere length remained unchanged or even increased slightly over time. In unadjusted analysis, T/S ratio was lower in hypertensive patients than in normotensive subjects (Table 1), but this was because hypertensive subjects were older (47.0 years vs. 59.1 years ($P < 0.0001$); Table 1). After adjustment for age, there was no difference in telomere length between normotensive and hypertensive subjects ($P > 0.70$). In our study, telomere length was not associated with any of the anthropometric measurements, including body mass index analyzed as a continuous ($P \geq 0.36$) or categorical ($P = 0.46$) trait.

Association between LV phenotypes and telomere length

We analyzed the association between LV phenotypes and telomere length in multivariable-adjusted analyses while accounting for sex, age, body weight and height, systolic blood pressure, and use of antihypertensive drugs. There was a significant positive association of both LV mass and LV mass index at the follow-up examination with T/S ratio at baseline in all subjects and in the normotensive subgroup (Table 2). In all participants, LV mass and LV mass index were 5.20 g ($P = 0.003$) and 2.70 g/m^2 ($P = 0.004$) greater, respectively, for a 1-SD increment in the T/S ratio (approximately 0.75 kilobase pairs). In the normotensive subgroup, the corresponding effect sizes were 8.03 g ($P = 0.0001$) and 3.74 g/m^2 ($P = 0.0007$), respectively, but the associations were not significant in the hypertensive subgroup (Table 2). There were corresponding associations with LV wall thickness, particularly noticeable in the normotensive subjects, but no association with LV internal diameter (Table 2). These findings were consistent when we used the residuals of LV mass and the T/S ratio to remove the effect of age and anthropometric characteristics rather than to adjust for these covariables in multiple regression analyses (Figure 2). The cross-sectional and multivariable-adjusted associations between the LV traits and the T/S ratio at follow-up

Table 1. Characteristics of Subjects Included in a Study of Telomere Length and Left Ventricular Mass, Belgium, 1996–2007

Characteristic	All Subjects (n = 334)			Normotensive Subjects (n = 198)			Hypertensive Subjects (n = 136)			P Value
	No.	%	Mean (SD)	No.	%	Mean (SD)	No.	%	Mean (SD)	
Female sex	181	53.2		106	53.5		71	52.2		0.81
Age, years			51.9 (14.3)			47.0 (13.5)			59.1 (12.2)	<0.0001
Body weight, kg			74.4 (12.9)			72.1 (12.3)			77.7 (13.0)	<0.0001
Body height, cm			168.1 (9.0)			169.0 (8.9)			166.7 (9.02)	0.18
Body mass index[a]			26.3 (3.8)			25.2 (3.6)			27.9 (3.5)	<0.0001
Waist:hip ratio			0.86 (0.077)			0.84 (0.075)			0.89 (0.072)	<0.0001
Systolic blood pressure, mm Hg			129.7 (17.1)			119.5 (10.0)			144.5 (14.2)	<0.0001
Diastolic blood pressure, mm Hg			80.0 (9.2)			75.9 (6.8)			85.8 (9.1)	<0.0001
Smoking	71	21.3		53	26.8		18	13.2		0.003
Alcohol drinking	140	41.9		82	41.4		58	42.6		0.82
Total calorie expenditure, kcal/day			1,875 (599)			1,840 (593)			1,924 (605)	0.21
Hypertension[b]	136	40.7					136	100		
Treatment for hypertension	74	22.2					74	54.4		
T/S ratio[c] at baseline (1996–2000)			1.82 (0.26)			1.84 (0.27)			1.78 (0.26)	0.03
T/S ratio at follow-up (2005–2007)			1.76 (0.26)			1.80 (0.26)			1.71 (0.25)	0.003
T/S annual attrition rate			0.0077 (0.018)			0.0063 (0.019)			0.0097 (0.016)	0.10
Echocardiography										
LV internal end-diastolic diameter, mm			49.6 (4.8)			49.9 (4.7)			49.3 (5.1)	0.28
Interventricular septum, mm			10.0 (1.8)			9.4 (1.6)			10.8 (1.8)	<0.0001
Posterior wall, mm			8.9 (1.4)			8.5 (1.3)			9.6 (1.3)	<0.0001
Relative wall thickness			0.38 (0.076)			0.36 (0.067)			0.42 (0.76)	<0.0001
LV mass, g			169.9 (45.9)			158.9 (41.7)			185.9 (47.2)	<0.0001
LV mass index[d], g/m^2			91.8 (20.1)			86.6 (18.0)			99.3 (20.7)	<0.0001
LV hypertrophy[e]	28	8.4		4	2.0		24	17.6		<0.0001
LV concentric remodeling[f]	36	10.8		12	6.1		24	17.6		0.0008
Ejection fraction[g]			68.8 (7.8)			67.7 (7.6)			70.5 (7.8)	0.0007

Abbreviations: LV, left ventricular; SD, standard deviation.

[a] Weight (kg)/height (m)^2.

[b] Hypertension was defined as a blood pressure of at least 140 mm Hg (systolic) or 90 mm Hg (diastolic) or the use of antihypertensive drugs at baseline, follow-up, or both.

[c] T/S ratio, relative ratio of telomere repeat copy number (T) to single-copy gene copy number (*36B4* gene; S).

[d] LV mass index was defined as LV mass divided by body surface area, calculated as body weight$^{0.425}$ (in kg) × body height$^{0.725}$ (in cm) × 0.007184.

[e] LV hypertrophy is an LV mass index exceeding 110 g/m^2 in women and 125 g/m^2 in men. P values relate to the significance of the differences between normotensive and hypertensive participants.

[f] LV concentric remodeling was defined as a relative wall thickness exceeding 0.43.

[g] Relative volume difference (%) during the cardiac cycle.

(2005–2007) were somewhat attenuated in comparison with the associations seen with baseline telomere length, but trends were similar and the positive association in the normotensive subgroup remained highly significant (Table 3). However, as Table 3 shows, when we additionally included the longitudinally measured telomere attrition rate in the multivariable-adjusted models, the confidence intervals for the association between LV wall thickness or LV mass and the T/S ratio at follow-up narrowed. In all subjects, the parameter estimates for a 1-SD increase in the telomere attrition rate were −0.17 mm (95% CI: −0.29, −0.052; P = 0.005) for LV wall thickness and −4.27 g (95% CI: −7.49, −1.05; P = 0.009) for LV mass. In normotensive subjects, these estimates were −0.17 mm (95% CI: −0.31, −0.025; P = 0.02) and −4.09 g (95% CI: −8.37, −1.58; P = 0.004), respectively.

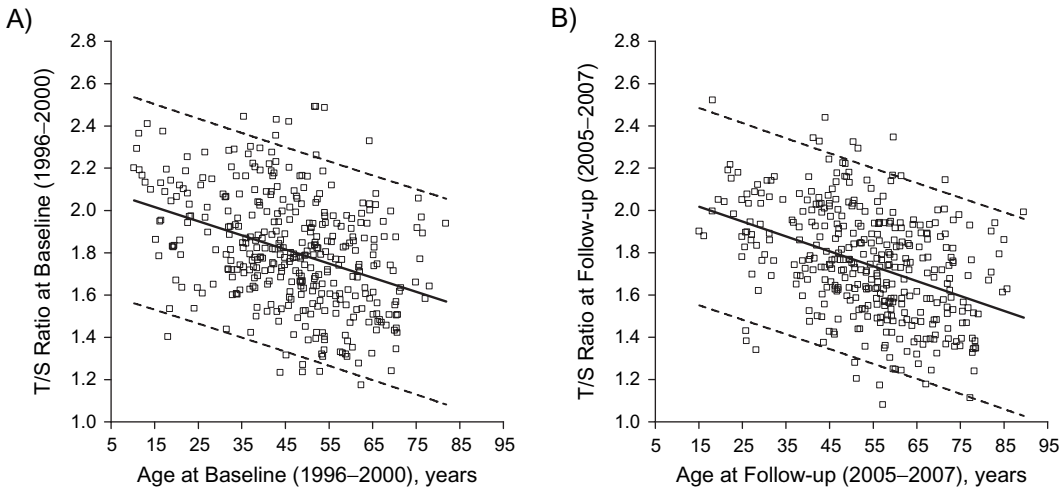

Figure 1. Relation between the relative ratio of telomere repeat copy number (T) to single-copy gene copy number (*36B4* gene; S) (T/S ratio) and age at A) baseline (1996–2000) ($n = 334$; $y = 2.12 - 0.0070 \times$ age; $P < 0.0001$) and B) follow-up (2005–2007) ($n = 334$; $y = 2.13 - 0.0068 \times$ age; $P < 0.0001$), Belgium. Dotted lines mark the 95% confidence interval for the prediction of the T/S ratio based on age in individual subjects.

DISCUSSION

The key finding of our study was that leukocyte telomere DNA length was associated with LV mass both longitudinally and cross-sectionally. To our knowledge, our study is the first population-based study demonstrating in a prospective manner an association between LV mass and telomere length. The median interval between the baseline telomere length measurement and the echocardiographic examination was 7.4 years. The association was stronger in normotensive subjects. Along similar lines, Vasan et al. (23) recently reported a positive cross-sectional association between multivariable-adjusted LV mass and telomere length measured by Southern blot analysis in 850 Framingham Heart Study participants. In our study, the estimated and measured annual decreases in T/S ratio were 0.0070 and 0.0080, which is in good agreement with the attrition rates observed in other populations measured with this technique (24, 25). Using the relation between polymerase chain reaction-based and Southern blot measurements of telomere length (20, 21, 24), this corresponds to 10–37 base pairs of telomere sequence per year.

Our findings are consistent with and extend to population-level previous work linking myocardial biology with telomere dynamics. It is now clear that there is a continuous regeneration of cardiomyocytes throughout life mediated by

Table 2. Multivariable-Adjusted Associations Between Left Ventricular Traits and T/S Ratio[a] Measured at Baseline (1996–2000), Overall and by Hypertension Status, Belgium, 1996–2007

Echocardiographic Characteristic at Follow-up (2005–2007)	T/S Ratio at Baseline (1996–2000)									*P*-Interaction[b]
	All Subjects ($n = 334$)			Normotensive Subjects ($n = 198$)			Hypertensive Subjects ($n = 136$)			
	Estimate[c]	95% CI	*P* Value	Estimate	95% CI	*P* Value	Estimate	95% CI	*P* Value	
Interventricular septum, mm	0.165	−0.005, 0.335	0.06	0.263	0.062, 0.465	0.007	0.029	−0.273, 0.328	0.85	0.27
Posterior wall, mm	0.128	0.001, 0.258	0.05	0.240	0.073, 0.406	0.004	0.015	−0.200, 0.224	0.88	0.21
LV diastolic diameter, mm	0.181	−0.265, 0.627	0.24	0.312	−0.244, 0.884	0.26	0.055	−0.562, 0.788	0.88	0.46
LV mass, g	5.20	1.81, 8.61	0.003	8.03	4.08, 12.0	0.0001	2.06	−4.26, 8.40	0.52	0.12
LV mass index[d], g/m^2	2.70	0.88, 4.50	0.004	3.74	1.77, 5.88	0.0007	0.905	−2.51, 4.32	0.59	0.19

Abbreviations: CI, confidence interval; LV, left ventricular.

[a] T/S ratio, relative ratio of telomere repeat copy number (T) to single-copy gene copy number (*36B4* gene; S).

[b] Significance of the interaction between T/S ratio and hypertension status.

[c] Estimates are expressed for a 1-standard-deviation (0.26) higher T/S ratio. Estimates for LV traits were adjusted for sex, age, body weight and height (not applicable to LV mass index), waist:hip ratio, total daily calorie expenditure, systolic blood pressure, and use of antihypertensive drugs (not applicable to normotensive subjects).

[d] LV mass index was defined as LV mass divided by body surface area, calculated as body weight$^{0.425}$ (in kg) $\times$ body height$^{0.725}$ (in cm) $\times$ 0.007184.

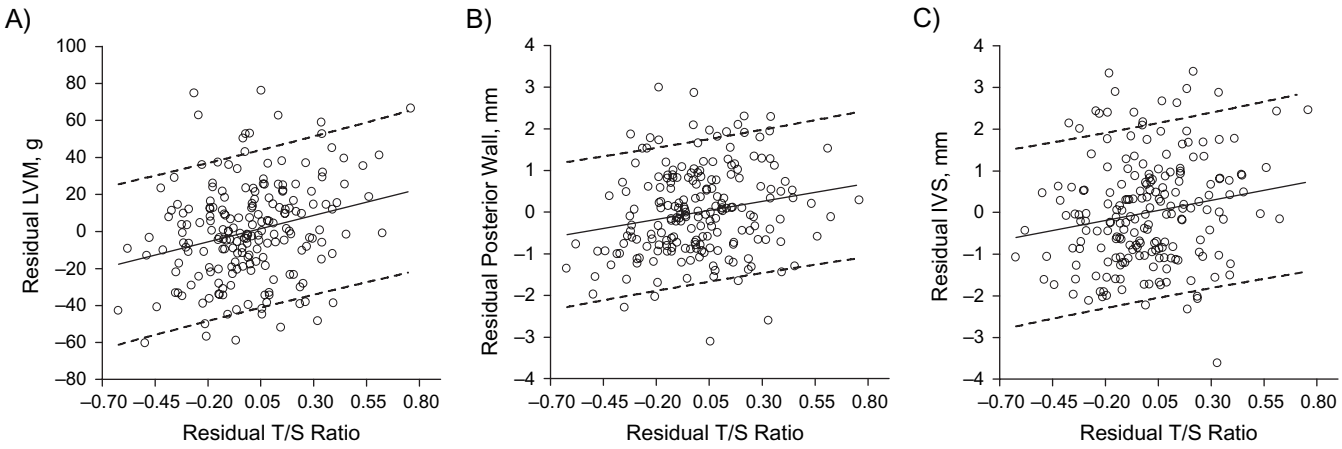

Figure 2. Correlations of the residuals of A) left ventricular mass (LVM) ($r = 0.26$, $P = 0.0002$), B) the posterior wall ($r = 0.20$, $P = 0.0057$), and C) the interventricular septum (IVS) ($r = 0.18$, $P = 0.011$) at follow-up (2005–2007) with the residuals of relative ratio of telomere repeat copy number (T) to single-copy gene copy number (*36B4* gene; S) (T/S ratio) at baseline (1996–2000) in normotensive subjects, Belgium. Residuals were computed to remove the variance explained by age and anthropometric characteristics. Dotted lines mark the 95% confidence interval for the prediction of left ventricular traits based on the T/S ratio in individual subjects with the effects of age and anthropometric characteristics removed.

a selected group of non-terminally differentiated cells (26). Such cells demonstrate telomere shortening and a senescent phenotype with age (27). In hearts from aged rats, Kajstura et al. (9) found that approximately 16% of cardiomyocytes showed telomere shortening.

More direct evidence for involvement of telomeres in cardiomyocyte size and regeneration comes from experimental manipulation of telomere length of cardiomyocytes. Telomere shortening in second- and fifth-generation telomerase knockout mice was accompanied by attenuation in cardiomyocyte proliferation, increased apoptosis, and cardiomyocyte hypertrophy (10). These cell impairments are concomitant with LV dilation, thinning of the wall, reduction of LV mass, and cardiac dysfunction in the later (fifth) generation of telomerase knockout mice (10). On the other hand, forced expression of TERT in cardiac muscle in mice was sufficient to rescue telomerase activity and telomere length and delay cardiac cell-cycle exit (12). In the first month after birth, the left ventricle in these mice was hypercellular, with increased myocyte density and DNA synthesis (12). By 12 weeks, cell cycling subsided; instead, cell enlargement (hypertrophy) was seen (12), but without fibrosis or impaired function, unlike other forms of induced cardiac growth (28). Thus, we speculate that under normal circumstances, subjects with longer telomeres might have larger cardiomyocytes and better regenerative capacity and hence maintain LV mass better. The finding that a greater LV posterior wall thickness was associated with a lower rate of telomere attrition from baseline to follow-up is consistent with this hypothesis. However, we cannot exclude the possibility that the association between LV mass and telomere length is not direct but is mediated by an unmeasured third factor which influences both LV mass and telomere length, such as oxidative stress or physical activity (29).

The above interpretation of the association between telomere length and LV mass, while consistent with experimental observations, is based on the assumption that mean leukocyte telomere length reflects the telomere length of cardiomyocytes. This remains to be directly shown. Nonetheless, telomere length has a strong genetic determination (30–32). Several studies have shown high intraindividual correlation of mean telomere length in different tissues, including leukocytes and vascular tissues (33, 34). In our study, the association between LV mass and telomere length was particularly significant in subjects who were normotensive at baseline and follow-up and in whom long-standing hemodynamic factors or treatment was unlikely to mask this association.

Pathologic cardiac growth induced by mechanical load (blood pressure) is characterized by enlargement and disorganization of cardiomyocytes, interstitial fibrosis, "fetal" cardiac gene induction, and apoptosis (28). In contrast, physiologically increased LV mass is associated with normal cardiac structure and normal or enhanced cardiac function (35). LV hypertrophy is associated with a poor prognosis and increased cardiovascular mortality and morbidity (19). In our normotensive group, in which we found a strong positive association between LV mass and T/S ratio, only 4 subjects (2.0%) had mild eccentric LV hypertrophy (mainly due to an increase in LV diastolic diameter). Thus, in our normotensive subjects, LV mass was distributed within a clinically normal (physiologic) range. However, even within this range, we demonstrated significantly different values of LV mass across quartiles of T/S ratio (Appendix Table 1). It is notable that in the study by Vasan et al. (23), the positive association between LV mass and telomere length was stronger in hypertensive subjects with pathologic LV hypertrophy than in normotensive subjects. The Framingham investigators did not provide an explanation for this somewhat unexpected finding, especially since some investigators have reported shorter telomeres in subjects with hypertension (36). The reasons for the difference in this regard between their observations and ours remain to be clarified.

Table 3. Multivariable-Adjusted Associations Between Left Ventricular Traits and T/S Ratio[a] Measured at Follow-up (2005–2007), Overall and by Hypertension Status, Belgium, 1996–2007

Echocardiographic Characteristic at Follow-up (2005–2007)	T/S Ratio at Follow-up (2005–2007)									*P*- Interaction[b]
	All Subjects (n = 334)			Normotensive Subjects (n = 198)			Hypertensive Subjects (n = 136)			
	Estimate[c]	95% CI	*P* Value	Estimate	95% CI	*P* Value	Estimate	95% CI	*P* Value	
Interventricular septum, mm										
Model 1[d]	0.109	−0.065, 0.283	0.22	0.203	0.005, 0.416	0.05	−0.007	−0.320, 0.304	0.96	0.35
Model 2[e]	0.146	−0.031, 0.611	0.11	0.250	0.039, 0.304	0.021	0.003	−0.312, 0.338	0.98	
Posterior wall, mm										
Model 1	0.042	−0.091, 0.175	0.53	0.161	−0.007, 0.328	0.06	−0.091	−0.315, 0.130	0.41	0.15
Model 2	0.086	−0.047, 0.224	0.20	0.213	0.042, 0.390	0.016	−0.055	−0.278, 0.169	0.62	
LV diastolic diameter, mm										
Model 1	0.112	−0.343, 0.567	0.63	0.195	−0.369, 0.762	0.49	0.028	−0.731, 0.785	0.94	0.66
Model 2	0.130	−0.338, 0.598	0.58	0.250	−0.343, 0.842	0.41	0.034	−0.728, 0.806	0.93	
LV mass, g										
Model 1	3.33	−0.20, 6.86	0.06	5.85	1.79, 9.88	0.005	0.018	−6.32, 6.82	0.93	0.12
Model 2	4.29	0.70, 7.85	0.019	7.28	3.15, 11.4	0.0007	0.874	−5.82, 7.57	0.79	
LV mass index[f], g/m^2										
Model 1	1.70	−0.20, 3.59	0.08	2.75	0.56, 5.00	0.01	0.208	−3.33, 3.74	0.91	0.16
Model 2	2.13	0.21, 4.06	0.029	3.35	1.11, 5.59	0.004	0.554	−3.02, 4.13	0.75	

Abbreviations: CI, confidence interval; LV, left ventricular.

[a] T/S ratio, relative ratio of telomere repeat copy number (T) to single-copy gene copy number (*36B4* gene; S).

[b] Significance of the interaction between T/S ratio and hypertension status.

[c] Estimates are expressed for a 1-standard-deviation (0.26) higher T/S ratio.

[d] In model 1, estimates for LV traits were adjusted for sex, age, body weight and height (not applicable to LV mass index), waist:hip ratio, total daily calorie expenditure, systolic blood pressure, and use of antihypertensive drugs (not applicable to normotensive subjects).

[e] In model 2, estimates were additionally adjusted for the longitudinally assessed telomere attrition rate.

[f] LV mass index was defined as LV mass divided by body surface area, calculated as body weight$^{0.425}$ (in kg) × body height$^{0.725}$ (in cm) × 0.007184.

The association between LV mass and telomere length at follow-up seemed attenuated in the cross-sectional analysis as compared with the analysis with telomere length measured on average 7.4 years earlier. Although this may simply reflect chance (as the effect sizes observed in the normotensive group at the 2 time points were not statistically different), it may also reflect a natural gradual exhaustion of the regenerative capacity provided by longer telomeres with increasing age. Therefore, just as in the case of hypertensive subjects, where earlier increased replicative stress might explain the loss of any association between LV mass and telomere length, increasing age would also tend to attenuate this relation. We noticed that in the last age quartile (>61.5 years), the association between LV mass and T/S ratio was significantly attenuated (Appendix Table 2). The finding that a lower rate of telomere attrition from baseline to follow-up was associated with greater LV wall thickness and LV mass is consistent with this hypothesis.

Given the importance of LV mass as a determinant of LV function, our finding that mean leukocyte telomere length predicts LV mass has potential clinical relevance. Indeed, in very elderly patients, mean leukocyte telomere length has been shown to be positively correlated with ejection fraction, with 1-SD-longer telomeres being associated with a 5% higher ejection fraction (37). Conversely, other studies have shown that mean leukocyte telomere length is shorter in patients with chronic heart failure than in age-matched controls (38). Furthermore, the severity of heart failure is inversely correlated with telomere length (38). This suggests that exhaustion of the replicative capacity within the heart as a consequence of shorter telomeres may be involved in the progression to heart failure and may determine its severity. Direct myocardial studies corroborate this possibility. Using confocal microscopy, Chimenti et al. (8) compared endomyocardial biopsies from 19 elderly patients with dilated myopathy with specimens from 7 aged-matched subjects with normal LV function. Myocytes from the diseased hearts showed a 39% reduction in average telomere length and a higher level of cell apoptosis and necrosis compared with specimens from healthy controls (8). Along similar lines, Oh et al. (39) demonstrated that dilated failing human hearts have telomeres that are 25% shorter than those of aged-matched controls, with decreased expression of telomere repeat-binding factor 2 and marked activation of DNA

damage response kinase (checkpoint kinase 2). Taken together, the findings indicate that telomere dynamics and their change with age and under stress play an important role in determining cardiac function.

The results in the present study must be interpreted within the context of its limitations and strengths. First, as discussed above, we measured telomere length in easily accessible circulating leukocytes; whether this measure reflects telomere length in cardiomyocytes remains to be confirmed. Second, variables reflecting LV structure are quantitative traits prone to measurement error. In the present study, 1 experienced observer performed all echocardiograms, with high intraobserver reproducibility.

In summary, longer mean leukocyte telomere length is associated with increased LV mass, particularly in normotensive subjects. The association could have a biologic basis related to the role of telomere length in determining cardiomyocyte replication. Whether the association can aid in risk stratification for heart failure and whether cardiac telomere dynamics can be manipulated for clinical benefit require further investigation.

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(Appendix tables follow)

Appendix Table 1. Multivariable Left Ventricular Traits of Participants According to Quartile of T/S Ratio[a] Measured at Baseline (1996–2000), Overall and by Hypertension Status, Belgium, 1996–2007

Characteristic	Quartile of T/S Ratio (1996–2000)							
	1		2		3		4	
	Estimate[b]	95% CI	Estimate	95% CI	Estimate	95% CI	Estimate	95% CI
All subjects	(*n* = 82)		(*n* = 86)		(*n* = 83)		(*n* = 83)	
T/S ratio cutpoints	<1.64		1.64–1.79		1.80–1.99		≥2.00	
LV traits								
Interventricular septum, mm	9.8	9.5, 10.2	10.3	9.9, 10.6	10.1	9.8, 10.5	10.5	10.1, 10.8
Posterior wall, mm	9.0	8.7, 9.3	9.2	8.9, 9.5	9.2	8.9, 9.5	9.3	9.0, 9.6
LV diastolic diameter, mm	49.1	48.1, 50.1	49.0	48.0, 50.0	49.4	48.5, 50.4	49.4	48.4, 50.4
LV mass, g	163	156, 170	170	164, 177	174	167, 180	177	170, 184
LV mass index[c], g/m^2	88	84, 91	92	88, 96	93	90, 97	95	91, 99
Normotensive subjects	(*n* = 49)		(*n* = 49)		(*n* = 50)		(*n* = 50)	
T/S ratio cutpoints	<1.66		1.66–1.81		1.82–2.05		≥2.06	
LV traits								
Interventricular septum, mm	9.0	8.6, 9.4	9.5	9.1, 9.9	9.3	8.9, 9.6	9.8	9.4, 10.2
Posterior wall, mm	8.2	7.9, 8.6	8.6	8.3, 9.0	8.6	8.3, 9.0	8.8	8.5, 9.2
LV diastolic diameter, mm	50.0	48.8, 51.2	48.8	47.6, 50.0	50.2	48.9, 51.4	50.0	48.7, 51.3
LV mass, g	153	144, 161	155	147, 163	164	156, 172	170	161, 178
LV mass index, g/m^2	84	79, 88	84	80, 88	88	84, 92	91	89, 96
Hypertensive subjects	(*n* = 33)		(*n* = 37)		(*n* = 33)		(*n* = 33)	
T/S ratio cutpoints	<1.64		1.64–1.79		1.80–1.99		≥2.00	
LV traits								
Interventricular septum, mm	11.0	10.4, 11.7	10.9	10.3, 11.5	11.0	10.3, 11.6	11.0	10.3, 11.6
Posterior wall, mm	9.7	9.3, 10.2	9.8	9.4, 10.2	9.7	9.2, 10.1	9.7	9.3, 10.2
LV diastolic diameter, mm	48.2	46.6, 49.8	49.1	47.6, 50.6	49.8	48.3, 51.4	48.7	47.1, 50.2
LV mass, g	181	168, 194	185	173, 197	190	177, 203	185	172, 198
LV mass index, g/m^2	97	90, 103	99	92, 105	102	95, 109	99	92, 106

Abbreviations: CI, confidence interval; LV, left ventricular.

[a] T/S ratio, relative ratio of telomere repeat copy number (T) to single-copy gene copy number (*36B4* gene; S).

[b] Estimates are expressed for a 1-standard-deviation (0.26) higher T/S ratio. Estimates were adjusted for sex, age, body weight and height (not applicable to LV mass index), waist:hip ratio, systolic blood pressure, use of antihypertensive drugs (not applicable to normotensive subjects), and daily calorie expenditure.

[c] LV mass index was defined as LV mass divided by body surface area, calculated as body weight$^{0.425}$ (in kg) × body height$^{0.725}$ (in cm) × 0.007184.

Appendix Table 2. Age-Stratified Multivariable-Adjusted Associations Between Left Ventricular Mass and T/S Ratio[a] Measured at Baseline (1996–2000) or Follow-up (2005–2007) in the Entire Study Population, Belgium, 1996–2007

T/S Ratio	Quartile of Age											
	1 (<43.7 Years) (*n* = 84; 75 (89.3%) Normotensive)			2 (43.7–52.2 Years) (*n* = 83; 55 (66.3%) Normotensive)			3 (52.3–61.5 Years) (*n* = 84; 41 (50.6%) Normotensive)			4 (>61.5 Years) (*n* = 83; 27 (32.5%) Normotensive)		
	Estimate[b]	95% CI	*P* Value	Estimate[b]	95% CI	*P* Value	Estimate[b]	95% CI	*P* Value	Estimate[b]	95% CI	*P* Value
Baseline (1996–2000)	3.77	−2.34, 9.91	0.22	3.87	−2.31, 10.1	0.21	10.1	2.65, 17.6	0.009	0.50	−8.48, 9.49	0.91
Follow-up (2005–2007)	1.54	−4.52, 7.62	0.61	3.43	−2.96, 9.88	0.29	7.88	−0.36, 16.1	0.06	−1.38	−10.5, 7.75	0.76

Abbreviation: CI, confidence interval.

[a] T/S ratio, relative ratio of telomere repeat copy number (T) to single-copy gene copy number (*36B4* gene; S).

[b] Estimates are expressed for a 1-standard-deviation (0.26) higher T/S ratio. Estimates were adjusted for sex, age, body weight and height, waist:hip ratio, systolic blood pressure, use of antihypertensive drugs (not applicable to normotensive subjects), and total daily calorie expenditure.